Ten Tips for Establishing a Successful Environmental Monitoring Program

By: Ziva Abraham

A successful environmental monitoring program is more than an exercise in sampling and collecting data. It requires a thoughtful, scientific approach which assesses the risk of microbial contamination to the product. Factors to consider range from regulations to identification of environmental isolates.

1. First and Foremost: Follow Regulations

The first point to consider when establishing an environmental monitoring (EM) program is whether the facility is classified per ISO 14644 or EU Annex 1 standards, or is a controlled environment where the rooms are not classified, but controlled to these standards. Often non-sterile and terminally sterilized product manufacturers will opt for the latter.

2. Design a Pristine Cleanroom

An important step in environmental control is to design a cleanroom or controlled environment which discourages microorganism growth. Walls and countertops should be smooth and easy to clean and disinfect. The disinfectant must meet efficacy standards and the cleaning procedure must be validated. Airflow should move from tightly classified rooms to lesser classified rooms. Equipment must be positioned so that it does not change the direction of the air. Appropriate gowning procedures must be written. Operators and cleanroom personnel must understand basics of microbiology and be trained in aseptic technique.

3. Know Your Product

In order to determine the risk of contamination for a particular product, several questions need to be answered. For beginners, is the product terminally sterilized, aseptically manufactured, low bioburden, or non-sterile? EM is important in all three cases because:

- If the product is terminally sterilized, the manufacturer needs to keep a product's pre-sterilization bioburden as low as possible. Air, water, personnel, and surfaces can contribute to the bioburden and thus need to be monitored.
- If the product is aseptically manufactured and purported to be sterile, no contamination is allowed. Adequate environmental control is critical to ensuring product sterility. In addition sterility testing must be conducted before release.
- If the product is a low bioburden, non-sterile product, there are two reasons for monitoring: the first is to keep product bioburden under control and the second is to know if the environmental isolates would be objectionable in the product via the mode of administration.

Other important questions to consider are: What is the antimicrobial effectiveness of the product? Will the product promote microbial growth? If so, which organisms can grow and circumvent the antimicrobials or pH controls?

4. Establish a Baseline

Before qualifying the cleanroom/controlled environment, it is advisable to get a baseline of total particulates and viable particulates in the air and on surfaces. This is both an indicator and a check to ensure the established limits will be met even after the facility is in operation. Establishing a baseline also allows for assessments of the types of organisms brought in during construction, and helps in developing a scientifically sound disinfection program to address chemical kill and physical removal of these contaminants. The data subsequently collected during microbiological performance qualification and routine monitoring helps to validate the efficacy of disinfection and cleaning procedures.

5. Pick Sampling Sites

The EM procedure should address the number and location of sampling sites. The number of sampling sites for total particulates can be calculated per the formula described in ISO 14644-1. Adopting a risk-based approach when choosing sampling sites provides a good picture of how the environment can affect the product. Hard to clean areas, areas with maximum activity, and areas close to critical operations are good examples of choosing site locations based on risk.

6. Choose Suitable Media

Tryptic Soy Agar (TSA) plates are commonly used for capturing bacterial growth and Sabouraud Dextrose Agar (SDA) for fungi. These plates, used as contact plates for viable air monitoring devices and/or for surface monitoring, usually contain lecithin and polysorbate 80 as neutralizers. It is important to note, however, these two neutralizers are only effective in neutralizing phenolic and quaternary ammonium compounds. If bleach or PAA chemistries are used for disinfection, they will not be neutralized and consequently, test results will be erroneous. Using only one medium that will successfully neutralize all disinfectants is an option.

7. Decide Incubation Process

Using one medium means the same plate needs to be incubated at dual temperatures (20-25°C and 30-35°C). At which temperature should the plate be incubated first, higher or lower? In my opinion, it is best to capture the bacteria at 30-35°C, and then move the plate to 20-25°C for fungal growth. Reasons include:

- Bacteria are more delicate than fungal spores in structure
- Most fungi will grow at 30-35° C as well as 20-25°C
- Reading the plates for bacterial growth after two days of incubation will provide an opportunity to count mold colonies before they merge and form a mat of growth.

8. Pick an Identification System

Microbial identification is a very important piece of the environmental monitoring program. One cannot evaluate and remediate contamination without knowing where the contaminants are coming from. Identifying microbes provides good clues of their origin; soil, water, personnel or compromised structure. For example, *Bacillus* is associated with soil, *Pseudomonas* with water, and *Staphylococcus* with human skin. Microbial identification can be costly, therefore, good judgment must be exercised in regard to when and from where the recovered microorganisms will be identified. Only one representative colony of each type of isolate needs to be identified.

9. Data Trending

EM data must be continuously analyzed in order to assess risk to the product. Trending EM data, including types of microbes, allows manufacturers to determine if microorganism numbers are increasing, or if new organisms are turning up in the environment. Knowing the top ten predominant isolates assists in identifying their source, thus allowing appropriate measures to be taken in correcting deficiencies.

10. Preserve In-house Isolates

Regulators increasingly encourage the use of in-house isolates for testing disinfectants, growth promotion and other microbiology related tests. In-house isolates collected during environmental isolation can be preserved. Lyophilization, also called freeze-drying, is a useful method for long-term preservation which allows easy access to healthy cultures as needed for testing. Maintaining in-house isolates internally can be time consuming, labor-intensive, and problematic. Suppliers, such as Microbiologics, offer services in which you can have your in-house isolates professionally preserved and manufactured into convenient, ready-to-use formats, see below for more information.

Biography:

Ziva Abraham is the President and Founder of Microrite, Inc., a California based consulting firm providing consulting and training services to pharmaceuticals, biotechnology, medical devices and in vitro diagnostics in the areas of quality assurance, quality control, microbiology, and validation. Ziva has over 25 years of academic, research, clinical and industrial experience in microbiology, and quality assurance. Ziva has received her Master's Degree in microbiology and has conducted research on developing microbial Insecticides using entomogenous bacteria and fungi. Her career also includes founding and managing clinical laboratories for Maccabi Medical in Israel. She has trained personnel from various industries in microbiology techniques and methods. She uses her extensive experience to teach why assessing risk of microbial contamination should be in the forefront of any company that has products for human/veterinary use. Her experience in clinical laboratories has provided her with the framework to understand the effects of microbial contamination in products from a patient safety perspective.